Polymerase Chain Reaction (PCR)

PCR is used for amplification of DNA sequences for cloning and colony PCR.

Standard PCR:

Primers are designed manually to have a sequence-dependent melting temperature (TM) of 50-65 °C. For amplification of DNA-fragments Phusion High-Fidelity DNA Polymerase in FH buffer (Thermo Scientific) or Q5 polymerase is applied. The reaction mixture for Phusion High-Fidelity PCR Master Mix is composed as listed below (Table 1). The reaction mixture for Q5 Polymerase PCR Master Mix is composed as listed below (Table 2).

Table 1: composition of reaction mixture for Phusion High-Fidelity Master Mix

Compound	Concentration in reaction mixture	Volume in 20 µL reaction mixture
Nuclease free Water	-	x
2x Phusion HF Buffer	1x	10
Primer Forward	0.5 µM	1
Primer Reverse	0.5 µM	1
Template DNA	2-10 ng/µL	-
Phusion DNA Polymerase	-	0.5

Table 2: composition of reaction mixture for Q5 polymerase Master Mix

Compound	Concentration in reaction mixture	Volume in 25 µL reaction mixture
Nuclease free Water	-	x
5x Q5 Reaction Buffer	1x	5
10 mM dNTPs	200 µM each	0.5
Primer Forward	0.5 µM	1.25
Primer Reverse	0.5 µM	1.25
Template DNA	< 1000 ng	x
(5x Q5 High GC enhancer)	(1x)	(5)
Q5 DNA Polymerase	0.02 U/µL	0.5

Add all components in the provided order. Keep the samples on ice, before adding the polymerase vortex and centrifuge the samples. After the addition of the polymerase samples are shortly centrifuged and put in the PCR cycler, see below (Table 3 and Table 4).

Table 3 : PCR program for insert DNA amplification with Phusion polymerase

Step	Temperature / °C	Time / min	cycles
Initial denaturation	98	3:00	1
Denaturation	98	0:30	30
Annealing	x (primer dependent)	0:30	30
Extension	72	x (15-30 sec / 1kb)	30
End elongation	72	10:00	1
Hold	4	-	-

Table 4 : PCR program for insert DNA amplification with Q5 polymerase

Step	Temperature / °C	Time / min	cycles
Initial denaturation	98	0:30	1
Denaturation	98	0:10	30
Annealing	x (primer dependent)	0:30	30
Extension	72	x (20-30 sec / 1kb)	30
End elongation	72	2:00	1
Hold	4	-	-

After the amplification the DNA needs to be purified.